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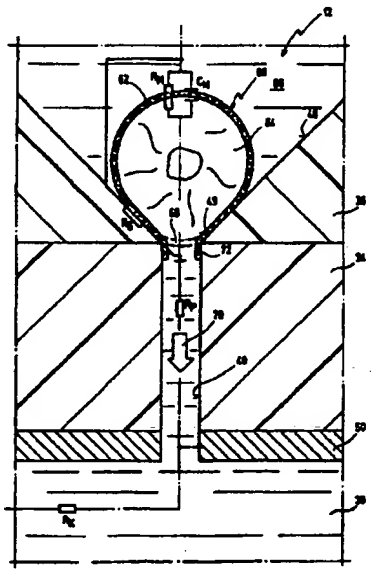
[Fortsetzung auf der nächsten Seite]

(54) Title: METHOD AND DEVICE FOR TAKING MEASUREMENTS OF CELLS WHICH ARE CONTAINED IN A LIQUID ENVIRONMENT

(54) Bezeichnung: VERFAHREN UND VORRICHTUNG ZUM MESSEN AN IN EINER FLÜSSIGEN UMGEBUNG BEFINDLICHEN ZELLEN



(57) Abstract: The invention relates to a method and a device for taking measurements of cells (60) which are contained in a liquid environment (66). According to the invention, the underside (68) of the membrane (62) of each cell (60) is positioned on a surface (48), said surface (48) being penetrated by a channel (40), in which a negative pressure (70) is created in order to attach the cell (60) by suction. The cell (60) is also electrically interrogated via at least one electrode (50), which is positioned at a distance from the cell (60). The negative pressure (70) is preferably set to pulse, in order to open the membrane (62) so that a connection is formed between the cell interior (64) which is surrounded by the membrane (62) and the channel (40).

(57) Zusammenfassung: Ein Verfahren und eine Vorrichtung dienen zum Messen an in einer flüssigen Umgebung (66) befindlichen Zellen (60), bei dem jede Zelle (60) mit einer Unterseite (68) ihrer Membran (62) auf einer Oberfläche (48) positioniert wird, wobei die Oberfläche (48) von einem Kanal (40) durchsetzt ist, in dem zum Ansaugen der Zelle (60) ein Unterdruck (70) eingestellt wird. Die Zelle (60) wird ferner über mindestens eine Elektrode (50) elektrisch abgefragt, die von der Zelle (60) beabstandet angeordnet ist. Der Unterdruck (70) wird vorzugsweise zum Aufreißen der Membran (62) pulsartig eingestellt, so daß das von der Membran (62) umschlossene Zellinnere (64) mit dem Kanal (40) verbunden wird.

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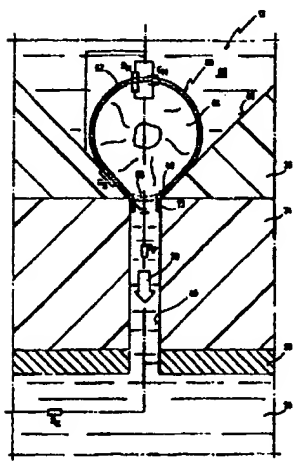
(54) Title: METHOD AND DEVICE FOR TAKING MEASUREMENTS OF CELLS WHICH ARE CONTAINED IN A LIQUID ENVIRONMENT



(57) Abstract: The invention relates to a method and a device for taking measurements of cells (60) which are contained in a liquid environment (66). According to the invention, the underside (68) of the membrane (62) of each cell (60) is positioned on a surface (48), said surface (48) being penetrated by a channel (40), in which a negative pressure (70) is created in order to attach the cell (60) by suction. The cell (60) is also electrically interrogated via at least one electrode (50), which is positioned at a distance from the cell (60). The negative pressure (70) is preferably set to pulse, in order to open the membrane (62) so that a connection is formed between the cell interior (64) which is surrounded by the membrane (62) and the channel (40).

Process and device for measurement on cells
located in a liquid environment

The invention relates to a process for measurement on cells located in a liquid environment, in which each cell is positioned with the bottom of its membrane on a surface, the surface being penetrated by at least one channel in which a pressure drop is established to suck the cell onto the surface and furthermore the cell is electrically interrogated via at least one electrode.

The invention furthermore relates to a device for electrical measurement on cells which are located in a liquid environment, with a substrate which is penetrated by a channel, above which a cell can be positioned with the bottom of its membrane on the surface of the substrate, there being means for producing a pressure drop along the channel and there being a first electrode for electrical interrogation of the cell.

A process and a device of the aforementioned type are known from DE 197 12 309 A1.

Use of so-called microelectrode arrangements to study biological cells is known. The microelectrode arrangements are used here for example to stimulate the cells or to discharge potentials. Studies can be done here in a biological environment or even in an artificial environment. The arrangements for this purpose comprise in a carrier body a plurality of microelectrodes, with dimensions which are roughly of the magnitude of the cells, therefore in the range from a few microns to a few dozen microns. A microelectrode arrangement of this general type is known for example from WO 97/05922.

In conventional microelectrode arrangements it is more or less random whether one or the other cell settles on a certain electrode or not. In practice the cells generally settle only with partial overlap on one electrode so that the stimulation of the cell or the discharge of the cell potential is limited to this partial surface. Moreover, the cells settle only loosely on the electrodes. This can lead to problems with respect to the sealing resistance of the reference electrode. Cells can also come to rest outside the area of an electrode so that they cannot be acquired during measurement.

In the microelectrode arrangement known from the initially mentioned DE 197 12 309 A1 these defects are avoided by the cells being captured in microcells with an electrode located on their bottom. The electrode is provided with a central channel in which a negative pressure can be produced via suitable connecting channels which run underneath the electrodes. In this way it is possible to pull individual cells purposefully onto the electrodes and fix them at a certain contact pressure on the electrodes. Then measurements can be taken on the electrodes, but only from their outside.

Sucking cells on a pipette with negative pressure is known from another specialty, so-called patch-clamp technology (compare the US journal "Nature, vol. 260, pages 799-801, 1976)). But in patch-clamp technology the pipette must be guided specifically to the individual cell. In patch-clamp technology the cells with which contact is to be made are not moved, since they are generally adhering to a substrate. Conventional contact-making with cells using patch-

clamp pipettes however has the disadvantage that the number of cells with which contact can be made at the same time is extremely limited, since for reasons of space an optionally large number of pipettes cannot be inserted into the culture chamber.

On the other hand, compared to the technology described above, in which measurements are only possible from the outside of the cell, patch-clamp technology has the advantage that the cell interior can also be included in the measurement.

In the conventionally used patch-clamp technology with individual pipettes, this is done under microscopic observation by the fragile glass pipette being guided by means of a micromanipulator to an individual cell which adheres to the substrate and by the membrane being carefully sucked onto the pipette opening. There is therefore direct contact between the glass surface and the membrane surface. In this way a membrane spot is sealed against the surrounding liquid and electrically insulated. This insulation is also called a "gigaseal". From this "cell-attached configuration" the so-called "whole-cell configuration" is reached by continuing to suck the sealed membrane. This takes place by a piece of the membrane being breached underneath the pipette. In this way hydraulically and electrically sealed access via the pipette opening to the cell interior is formed. The remaining cell membrane is thus electrically accessible as a whole (so-called "whole cell patch"). Use of this conventional method however requires a pertinent amount of experience and instinctive feel. Several cells can be processed only sequentially. This method is therefore unsuited for large-scale studies, such as for example would be necessary in the area of pharmascreening, substance screening and the like.

Therefore the object of the invention is to develop a process and a device of the initially mentioned type such that the aforementioned disadvantages are avoided. In particular, the invention is intended to enable measurements as consistent as possible, preferably in parallel on a plurality of cells, especially as is desirable in the area of experimental and application-oriented screening of the action of pharmaceutical agents on the cellular level.

In a process according to the initially mentioned type this object is achieved as claimed in the invention in that the bottom of the membrane is torn open by increasing the pressure drop and/or the membrane is made microporous and electrically of low resistance or torn open by addition of pore-forming substances or by an electrical current pulse.

In a device according to the initially mentioned type the object of the invention is achieved in that a least a second electrode is spaced apart from the first electrode in the direction of the channel.

The object of the invention is fully achieved in this way.

The invention makes it possible, compared to conventional patch-clamp technology, to relinquish the difficult manipulation of a fragile glass pipette, since the function of the conventional glass pipette is performed by the channel in a substrate to which a negative pressure is applied in order to establish the cell-attached configuration. If a megaseal is established in this way between the cell wall and the surface onto which the cell is being sucked, as claimed in the invention either the bottom of the membrane is torn open by the increase of negative pressure, so

measurement electrode at a time.

A process is preferable in which the measurement quantity of the electrical signal is the current (I_{st}) through the cell interior and/or the electrical potential on the electrodes is measured.

In one preferred embodiment of the process as claimed in the invention the cell is electrically interrogated via an electrode which is spaced apart from the bottom of the membrane in the direction of the channel. To do this, a current can be injected into the cell interior.

This measure has the advantage that direct electrical access is established only into the interior of the cell. Because the cell with its outer membrane rest tightly on the bottom of the microcell and is even fixed there by negative pressure, the gigaseal known from the conventional patch-clamp technology is automatically formed, i.e. extremely high leak resistance which therefore has little effect on the measurement between the intracellular and extracellular medium. Because the electrode is located spatially away from the gigaseal, it is furthermore ensured that the cell itself is connected only to the electrically insulating materials so that the maintenance of the gigaseal is guaranteed.

In one preferred embodiment of the process as claimed in the invention, in an arrangement with a plurality of channels the pressure pulse or the electrical current pulse is applied at the same time to all channels. But alternatively it is also possible to apply the pressure pulses or current pulse in succession to individually selected channels, whether in any sequence or in a strictly sequential procedure.

These measures have the advantage that almost any experiments can be automatically carried out on a plurality of cells so that are very large number of measurement results are obtained each time.

In a preferred development of the process as claimed in the invention the composition of the intracellular liquid medium after opening the membrane or producing the micropores is changed by adding substances or the intracellular medium is replaced. To do this the channel can be connected to two or more separate connecting channels, one being filled with electrolyte which is similar in its composition to that of the cytoplasm (intracellular liquid) or which is a special liquid with active agents added.

In this way the intracellular medium can be influenced in a specific and controlled manner and at the same time the possible range of measurements which can be taken is greatly increased.

In the device as claimed in the invention there is at least a second electrode spaced apart from the first electrode in the direction of the channel.

In one preferred development there are means for controlling the pressure drop, both for producing a static pressure drop for establishing a cell-attached configuration, and also for a pulsed increase of the pressure drop to tear open the bottom of the membrane.

In this way, on the one hand the megaseal can be established in a reliable and controlled manner and on the other hand the megaseal can be maintained while a short pressure pulse tears open the bottom of the membrane and the bottom of the membrane rests against the channel wall. In doing so the control is preferably designed such that the static negative pressure is continuously maintained (offset pressure) so that the megaseal is also maintained in the whole-attached configuration.

According to another embodiment of the device as claimed in the invention, the electrode is located on the end of the channel which faces away from the first electrode.

Here the electrode can annularly surround the end of the channel which faces away.

These measures have the advantage that the electrode can be easily integrated into a microstructure by its being formed on the bottom of the layer in which the channel runs.

In another version of the invention the electrode is conversely located at a distance from the end of the channel which faces away.

This measure has the advantage that the electrode, as is yet to be explained, can also be movable relative to the channel so that several cells can be measured in succession with the same electrode.

According to another feature of the invention the channel on its end facing away from the first electrode is connected via valves to a plurality of channels via which liquid can be supplied and drained.

This measure has the advantage that by the corresponding monitoring of the pressure conditions in the connecting channels the cell interior after forming the whole-cell configuration, i.e. after breaching the membrane, comes into contact with the intracellular liquid.

In an additional development of the invention, above the substrate there is a microcell with an opening in its bottom.

In this way the liquid can be stored above the substrate in a manner which is suitable especially for a large-scale study by there being a channel or channels. Here the cells can be routed by a suitable funnel-shaped execution of the microcell directly into the vicinity of the channel mouth of the substrate surface. Alternatively the opening in the bottom of the microcell however can also have a much larger diameter so that the cell is guided to the mouth of the channel essentially by the applied negative pressure. This facilitates the production of the structure as claimed in the invention.

Furthermore it is preferred that a plurality of channels be located in a common substrate.

In this way a compact construction can be achieved with simple production.

Furthermore, there is preferably a plurality of microcells in a plate.

This yields the advantage that parallel or sequential measurements can be easily prepared on many cells because all microcells are located in a common plate.

In embodiments of the invention which use a common plate for the microcells, it is furthermore preferred if the plate is built up of several layers.

This measure has the advantage of being able to take into account the different demands on the different elements of the plate by suitable material choice.

This applies especially when the plate comprises a top layer, a middle layer and a bottom layer in a development of this version, in the top layer there being the microcells, the middle layer forming the substrate with the channels, and in the bottom layer there being connecting channels which lead to the channels, and in one preferred embodiment there are electrical supply lines which lead to the channels, and microelectrodes.

This three-part division of the plate has the advantage that individual layers of varied thickness and varied materials can be used for each of the three important functions.

According to another version of the invention the substrate is connected to the bottom layer which consists of one or more layers of photostructurable materials which allow three-dimensional routing of the connecting channels which lead to channels.

Here the bottom layer can be applied to a glass carrier.

These measures enable an especially compact construction and simple production. Photostructurable materials include certain polymers, but also certain glasses.

It is preferred if the connecting channels have a width between 10 microns and 40 microns, preferably roughly 20 microns.

The channels themselves preferably have an inside diameter of less than 10 microns, preferably less than 5 microns.

These amounts have proven optimum in this connection. The positioning of one cell at a time on one channel is supported especially by the inside diameter of the channels being less than the cell diameter.

As already indicated above, in these embodiments of the invention it is especially preferred if the electrodes are located on the bottom of the middle layer or the top of the bottom layer.

This measure has the advantage that the electrodes together with their supply lines can be formed by simple impression, precipitation, vapor deposition and subsequent microstructuring by known processes (photolithography, etching processes, lift-off, etc.).

It is furthermore preferred in this case if the electrodes in a plan view are made as a

square surface with an edge length between 20 and 60 microns, preferably roughly 40 microns.

As already mentioned, in this case it can be provided that the printed conductors leading to the electrodes are located between the middle and the bottom layer. This can be done alternatively by their being applied underneath to the middle layer or at the top to the bottom layer. Application to the bottom of the middle layer has the advantage that the printed conductors can be formed together with the electrodes, especially also with the same material, especially a precious metal, preferably gold.

The printed conductors preferably have a width between 5 microns and 30 microns, especially roughly 10 microns.

As was already mentioned, different materials can be used for the individual layers in a multilayer construction of the plate.

The different layers can be produced independently of one another, for example, from plastic, polymethyl methacrylate (PMMA), silicone, PTFE, polyamide or an inorganic material, especially from glass, ceramic or silicon.

Preferably polyamide is used for the substrate; it is used for this purpose as a film in which the channels are formed as holes. The substrate (the film) then preferably has a thickness between 2 microns and 40 microns, preferably roughly 5 microns.

Preferably glass is used for the bottom layer. In glass which can be made available in almost any thickness, in order to ensure mechanical stability in this way the necessary connecting channels and the like can be formed in the conventional manner.

In an additional development of the invention it is preferred that the substrate be located on the bottom of a plate in which a plurality of holes are formed as microcells, with holes in their bottom, with which holes the channels of the substrate are centered.

In this way a combined body with a plurality of microcells to which individual channels and electrodes are assigned can be relatively easily produced.

According to another embodiment of the invention there is a hydraulic and measurement unit which has a chamber which is open to the bottom of the substrate and which can be positioned on the bottom of the substrate such that the chamber communicates with a selected channel and is sealed to the outside, the chamber containing at least one electrode and being connectible to at least one connecting channel which is joined to the negative pressure source.

In this case there can furthermore be a movement unit for moving and positioning the plate and the negative pressure and measurement unit relative to one another.

In this way a single measurement unit can be used for sequential measurement of a plurality of cells, by which major cost savings can result.

Preferably commercial, ordinary standard grid plates (so-called "96 hole plates", "384 hole plates or the like) can be used. They require simply to be closed at the bottom by applying the film which is provided with channels (holes). The measurements on the numerous individual cells in the holes of the perforated plate are then subsequently taken in sequence by moving the negative pressure and measurement unit or vice versa by moving the standard grid plate with reference to the stationary negative pressure and measurement unit. The latter produces the negative pressure pulse for opening the cell and also contains the electrode in order to take the subsequent measurement through the cell interior.

In turn, as already mentioned, the chamber can be connected via valves to a plurality of connecting channels in order to bring the cell interior after formation of the whole-cell configuration into contact with intracellular liquid or to be able to change the composition of the intracellular liquid.

Other advantages follow from the specification and the attached drawings.

It goes without saying that the aforementioned features and those still to be explained below can be used not only in the respectively given combination, but also in other combinations or alone without departing from the framework of this invention.

Embodiments of the invention are shown in the drawings and are detailed in the following description.

Figure 1 shows an extremely schematic perspective view of one embodiment of the device as claimed in the invention;

Figure 2 shows a section through a microcell of the arrangement shown in Figure 1, likewise highly schematic;

Figure 3 shows a plan view of the microcell as shown in Figure 2 on a slightly reduced scale;

Figure 4 shows an extract from Figure 2, on a further enlarged scale, in explanation of the process as claimed in the invention;

Figure 5 shows a representation, similar to Figure 3, showing the prior art;

Figure 6 shows a perspective view and an enlarged extract from it, of another embodiment of a device as claimed in the invention; and

Figures 7a) to 7c) show different phases in the sucking of a cell, the formation of the cell-attached configuration and the whole-cell configuration when using two connecting channels to the channel, in a simplified schematic representation.

Example 1

In Figures 1 to 4 the plate is labeled 10. In the surface 11 of the plate 10 a grid of microcells 12 is formed by molding. The microcells 12 are three-dimensional and have dimensions suitable for the culture of cells. For example, there can be 8 x 12 = 96 microcells 12 in one plate 10.

The arrow 14 indicates that the microcells 12 can be filled from above with a liquid containing the cells to be studied. It is thus possible to fill the microcells 12 each individually with different liquids and cells.

To take the measurements there is an electrical terminal module 16 which can be coupled laterally to the plate 10, for which there is a sufficient number of connectors 18. The connectors 18 are connected to a network of printed conductors. These printed conductors lead to electrodes which are attached in the area of the microcells 12, as will be explained below. A data line 20 leads to a control device 22 from the electrical terminal module.

Furthermore, there is a hydraulic connection module 24 which can likewise be coupled laterally to the plate 10 by means of a corresponding plurality of hydraulic connectors 26. A negative pressure can be produced via the hydraulic connecting module 24 in a predetermined manner, especially individually, underneath the microcells 12, especially pulsed over time, as will be explained in detail below.

For this purpose the hydraulic connectors 26 are connected via a network of connecting channels and openings 49 on the bottom of the microcells 12 to the latter. When all microcells 12 are to be exposed to the same negative pressure, all connecting channels are connected in parallel and are directly connecting in the hydraulic connecting module 24 to a central, controlled negative pressure source. If however the individual microcells 12 are to be triggered with a respectively individual negative pressure, likewise a central negative pressure source can be used which is connected to the network of connecting channels, then individually controllable valves being located in these connecting channels. But alternatively in the connecting channels there can also be miniaturized pumps, especially miniature membrane pumps which are individually triggered. The electrical triggering of the valves and/or the miniature pumps can be done either via the electrical terminal module 16 or the hydraulic connecting module 24. In each case a line 28 for triggering the aforementioned elements leads from the hydraulic connecting module 24 to the control device 22.

The control device 22 for its part is connected to a multiplexer 30 in order to be able to take several measurements at the same time or optionally in sequence in a predetermined manner.

As is apparent from Figure 2, the plate 10 consists essentially of three layers. On the bottom layer 32 is the middle layer or a substrate 34 which is made as a film. A top layer 36 is made as a microstructure layer. The bottom layer 32 consists preferably of glass. The substrate 34 is preferably a polyamide film. The microstructure layer 36 consists on the other hand preferably of polymethyl methacrylate (PMMA).

A connecting channel 38 is made in the top of the bottom layer 32. The connecting channel 38 is used for individual triggering of the microcell 12 which is shown in Figure 2. The connecting channel 38 is connected via a vertical channel 40 in the substrate 34 to an opening 49 on the bottom 48 of the microcell 12. The microcell 12 on its top is provided with a cylindrical section 42 which is lined with a reference electrode 44. The reference electrode 44 is connected to a first electrical terminal 46. The latter is preferably at ground.

Underneath, the cylindrical section 42 is adjoined by a funnel-shaped section which forms the bottom 48 in which there is an opening 49.

An electrode 50 is arranged roughly annularly around the lower end of the vertical channel 40. It is applied to the bottom of the substrate 34 for this purpose. The electrode 50 is connected to a supply line 52 which runs between the bottom layer 32 and the substrate 34. The supply line 52 for example together with the electrode 50 can be imprinted, vapor deposited, precipitated or the like on the bottom of the substrate 34. The supply line 52 is connected to a second electrical terminal 54.

The electrodes 46 and 50 consist of silver/silver chloride (Ag/AgCl). Technically these electrodes are called "reversible" or "non-polarizable". They have the advantage that not only AC voltage measurements, therefore measurements of potential spikes, but also DC voltage measurements are possible on the cells. They can also be used for current injection.

The measurement voltage U_{amp} is measured between the electrodes 46 and 50. Furthermore, a stimulation current I_{st} can be supplied via a second terminal 54 parallel to the voltage measurement.

This is detailed farther below using Figure 4.

As can be seen from the plan view as shown in Figure 3, there are several electrodes 12 in the plate 10 in the form of a grid, the grid pitch d being between 0.1 and 10 mm, preferably roughly 9 mm.

The microcells 12 in the area of their cylindrical section 42 have an inside radius r between roughly 1 and 9 mm, preferably roughly 7 mm,; this allows easy filling. The inside diameter x of the vertical channel 40 is less than 10 microns, preferably less than 5 microns.

The printed conductors 52 have a width b_1 between 5 microns and 30 microns, preferably roughly 10 microns. The electrodes 50 are preferably made square in a plan view and have an edge length a between 20 microns and 60 microns, preferably roughly 40 microns. The connecting channels 38 have a width b_2 between 10 microns and 40 microns, preferably roughly 20 microns.

The substrate 34 or the film is between 2 microns and 4 microns thick, preferably roughly 5 microns thick.

The distance l of the microcells 12 from the edge of the plate 10 is preferably at least 2

These holes 84 can be used as microcells. The plate 80 can, as indicated in Figure 6, top right, by the broken line, also be made in several layers, especially two layers.

The bottom 82 of the cylindrical holes or microcells 84 is formed by a substrate 86 which is made as a film and which is cemented, welded or otherwise bonded to the plate 80 on the bottom. In the center of the bottom 82 the substrate contains a channel 88 which is made as a hole.

In contrast to the embodiment as claimed in the invention as shown in Figures 2 to 4, underneath the substrate 86 there is no carrier with a system of connecting channels. Instead, there is a movable hydraulic and measurement unit 90 which can be guided individually from underneath to the bottom 91 of the film 86.

The unit 90 comprises a pot-shaped chamber 92 which is provided on its top face side with an annular seal 94. In this way the chamber 92 can be placed tightly against the bottom 91 such that the vertical axis of the chamber 92 is flush with the axis of one hole 88 at a time.

From the chamber 92 a pipeline 96 leads to a negative pressure unit which is not shown. In this way, in the chamber 92 a negative pressure can be produced as is indicated with an arrow 98.

On the bottom of the chamber 92 there is an electrode 100 which is connected to an external terminal 102.

A multiaxial movement unit is labeled 104. The movement unit 104 makes it possible to guide the hydraulic and measurement unit 90 along the bottom 91 from microcell 84 to microcell 84 in order to press the unit 90 from underneath tightly around the respective hole 88 against the bottom 91. By exposing the pipeline 96 to the negative pressure pulse 98 the same experiment can be carried out as was explained above using Figure 4 for the first embodiment of the invention.

The first embodiment of the invention as shown in Figures 2 to 4 has the advantage that a compact plate with all connecting channels is available for triggering the microcells 12 so that without additional actuating means, simply by triggering of valves, contacts and the like, a plurality of measurements can be taken in sequence or in multiplex operation.

The second embodiment as shown in Figure 6 conversely has the advantage that a commercial plate can be used and that the costs for a bottom layer with a plurality of connecting channels, printed conductors and individual electrodes are saved.

Example 3

Figures 7a) to c) show another embodiment of the invention in highly schematic form which is detailed below.

In turn, there is a substrate 110 which can consist for example of a polyamide film. In the substrate 110 a plurality of channels is formed, of which one which is labeled 122 is shown. Above the substrate 110 is a liquid in which there are cells 112. Underneath the channel 122 a chamber 124 is formed which communicates with the channel 122 and on its bottom there is an electrode 126 similarly to the version sh wn in Figure 6.

In contrast to the above described embodiments, this chamber 124 is however connected not only to one connecting channel, but to two connecting channels 130, 132. These connecting channels 130, 132 can be connected to the liquid reservoirs F_1 and F_2 via valves 118, 120.

It goes without saying that the representation is purely schematic and that the connecting channels 130, 132 can be made for example in a photopolyermerizable layer and that the valves are preferably made on the outer ends of the channels.

If at this point, as shown in Figure 7a), the valve 120 is closed and the valve 118 is opened, the application of a pressure P_1 to the channel 130 which is less than the pressure P_0 in the liquid 114 leads to formation of a flow in the direction of the arrow 133 through the channel 122 and the supply channel 130. This results in the cell 112 being sucked and coming to rest on the surface 128 of the substrate 110 above the opening of the channel 122 and a megaseal being formed when the negative pressure is maintained, so that the cell-attached configuration is established.

If at this point the valve 120 is opened as shown in Figure 7b), the relation $P_1 < P_2 < P_0$ being maintained, the chamber 124 is filled with intracellular medium from the liquid reservoir F_2, while a flow is established in the direction of the arrow 134 from the connecting channel 132 through the chamber 124 into the supply channel 130. Here the pressure P_2 must be greater than the pressure P_1 so that the flow in the direction of the arrow 134 is directed away from the connecting channel 132 toward the connecting channel 130; furthermore, the two pressures P_1 and P_2 must be less than the pressure P_0 in the extracellular medium 114 which surrounds the cell 112.

At this point, in the following phase as shown in Figure 7c) the valve 118 is closed and a pulsed negative pressure is applied to the connecting channel 132 so that P_2 is much, much less than P_0 ($P_2 << P_0$). In this way the bottom of the membrane of the cell 112, the membrane patch, is sucked and as a result of negative pressure shocks is broken open so that at this point a whole-cell-patch clamp is formed. Flow takes place during this phase in the direction of the arrow 135 through the channel 122 and the connecting channel 132 to the liquid reservoir F_2. At this point a state is reached in which the chamber 124 is filled solely with intracellular medium 116. Then via the valve 118 another medium can be used again if this is desired for the measurements to be taken.

The advantage of this arrangement and this process is that a precisely controlled intracellular medium with a composition which can be influenced can be used or that even another intracellular medium can be used.

This version with two or more connecting channels which can be controlled via valves

can basically be combined with the version which was explained above using Figures 1 to 4 and also with the version as sh wn in Figure 6.

Claims

1. Process for measurement on cells located in a liquid environment (66), in which each cell (50; 112) is positioned with the bottom (68) of its membrane (62) on a surface (48; 82; 128) which is penetrated by at least one channel (40; 88; 122) on which a pressure drop (70) is established to suck the cell (60; 112) onto the surface (48; 82; 128) and the cell (50; 112) is electrically interrogated via at least one electrode (44, 50; 100; 126), characterized in that the bottom (68) of the membrane (62) is torn open by increasing the pressure drop and/or the membrane (62) is made microporous and electrically of low resistance or torn open by addition of pore-forming substances or by an electrical current pulse.

2. Process as claimed in claim 1, wherein the pressure drop is increased in pulses to tear open the membrane (62).

3. Process as claimed in claim 1 or 2, wherein the bottom (48; 82) of the microcell (12; 84) is used to position the cell (60).

4. Process as claimed in one of the preceding claims, wherein the cell (60; 112) is electrically interrogated via an electrode (50; 100; 126) which is spaced apart from the bottom (68) of the membrane (62) in the direction of the channel (40; 88; 122).

5. Process as claimed in one of the preceding claims, wherein a current (I_{st}) is routed through the cell interior (64) or the potential is measured.

6. Process as claimed in one of the claims 1 to 5, wherein in an arrangement with a plurality of channels (40; 88; 122) the pulse (70) for increasing the pressure drop or the electrical current pulse for opening the membrane surface (68) is produced at the same time on all channels (40; 88; 122).

7. Process as claimed in one of the claims 1 to 5, wherein in an arrangement with a plurality of channels (40; 88; 122) the pulse (70; 98) for increasing the pressure drop or the electrical current pulse for opening the membrane surface (68) is produced in succession on selected individual channels or on several channels (40; 88; 122).

8. Process as claimed in one of the preceding claims, wherein the composition of the intracellular liquid medium (116) is changed by adding substances or the intracellular liquid medium (116) is replaced.

9. Device for electrical measurement on cells (60; 112) in a liquid environment (66), with a substrate (34; 86) which is penetrated by a channel (40; 88; 122), above which a cell (60; 112) can be positioned with the bottom (68) of its membrane (62) on the surface (49; 85; 128) of the substrate (34; 86), there being means (56) for producing a pressure drop (70) along the channel and there being a first electrode (44) for electrical interrogation of the cell (60; 112), characterized in that there is at least a second electrode (50; 100; 126) spaced apart from the first electrode (44) in the direction of the channel (40; 88; 122).

10. Device as claimed in claim 9, wherein there are means (56, 58) for controlling the pressure drop (70), both for producing a static pressure drop (70) for establishing a cell-attached configuration, and also for a pulsed increase of the pressure drop (70) to tear open the bottom (68) of the membrane (62).

11. Device as claimed in claim 9 or 10, wherein the second electrode (50) is located on the end of the channel (40) facing away from the first electrode (44).

12. Device as claimed in claim 11, wherein the second electrode (50) annularly surrounds the end of the channel (40) facing away.

13. Device as claimed in one of claims 9 to 12, wherein the channel (122) on its end facing away from the first electrode (44) is connected via valves (118, 120) to a plurality of channels (130, 132) via which liquid (F_1, F_2) can be supplied and drained.

14. Device as claimed in one or more of claims 9 to 13, wherein there is a plurality of channels (40; 88; 122) in a common substrate (34; 86; 110).

15. Device as claimed in one of claims 9 to 14, wherein over the substrate (34; 86) there is a microcell (12; 84) with an opening (49; 88) in its bottom (48; 82).

16. Device as claimed in one of claims 9 to 15, wherein the channel (40; 88; 122)) has an inside diameter (x) of less than 10 microns, preferably less than 5 microns.

17. Device as claimed in one of claims 14 to 16, wherein there is a plurality of microcells (12; 84) in a plate (10; 80).

18. Device as claimed in claim 17, wherein the plate (12; 80) is built up in several layers.

19. Device as claimed in claim 18, wherein the plate (10) comprises a top layer (36), a middle layer and a bottom layer (32), in the top layer (36) there being microcells (12), the middle layer forming the substrate (34) in which the channels (40) are located, and the bottom layer (32) containing connecting channels (38) which lead to the channels (40).

20. Device as claimed in one of the preceding claims, wherein the substrate (34; 86) is connected to the bottom layer (32) which consists of one or more layers of photostructurable materials which have connecting channels (38) which lead to the channels (40; 88; 122).

21. Device as claimed in claim 20, wherein the bottom layer (32) is applied to a glass carrier.

22. Device as claimed in claim 19, 20, or 21, wherein the connecting channels (38) have a width (b_2) between 10 microns and 40 microns, preferably roughly 20 microns.

23. Device as claimed in one of claims 19 to 22, wherein the electrodes are located on the bottom of the substrate (34) or on the top of the bottom layer (32).

INTERNATIONAL SEARCH REPORT

Inter ...nal Application No
PCT/EP 00/08895

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/487 G01N27/403

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 198 41 337 C (MICRONAS INTERMETALL GMBH) 23 September 1999 (1999-09-23)	9-12
Y	column 2, line 20 - line 48 column 3, line 8 - line 12 column 3, line 27 - line 44 column 4, line 27 - line 47 column 5, line 35 - line 41 column 6, line 33 - line 41 column 7, line 32 - line 51 column 8, line 36 - line 65 column 11, line 4 - line 23 column 12, line 28 - line 56	1-8,15, 17,18, 20,21, 23,27

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex

* Special categories of cited documents:

'A' document defining the general state of the art which is not considered to be of particular relevance

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'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 November 2000	12/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stussi, E

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/08895

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	E. NEHER AND B. SAKMANN: "Single channel currents recorded from membrane of denervated frog muscle fibers" NATURE, vol. 260, 1976, pages 799-802, XP000943702 London column 1 -column 2; figure 1	1,2,4-8
X,P	DE 198 27 957 A (MICRONAS INTERMETALL GMBH) 9 December 1999 (1999-12-09) column 1 -column 4 column 6, line 39 - line 50 column 15, line 68 -column 16, line 42; figure 22	1,2,4,5, 8-12
Y	DE 197 12 309 A (NMI NATURWISSENSCHAFTLICHES UN) 20 May 1998 (1998-05-20) cited in the application column 4, line 1 - line 10 column 5, line 48 - line 55 figures 1-3	3,15,17, 18,20, 21,23,27
A	US 5 262 128 A (LEIGHTON STEPHEN B ET AL) 16 November 1993 (1993-11-16) column 2, line 5 - line 41 column 3, line 54 - line 65 column 4, line 25 - line 34 column 4, line 56 -column 5, line 59	13-34
A	WO 97 05922 A (MISCH WILFRIED ;NMI NATURWISSENSCHAFTLICHES UN (DE)) 20 February 1997 (1997-02-20) cited in the application the whole document	1-34
A	EP 0 627 621 A (AVL MEDICAL INSTR AG) 7 December 1994 (1994-12-07) the whole document	1-34
A	EP 0 689 051 A (MATSUSHITA ELECTRIC IND CO LTD) 27 December 1995 (1995-12-27) the whole document	1-34
A	DE 196 46 505 A (ITT IND GMBH DEUTSCHE) 14 May 1998 (1998-05-14) the whole document	13-34

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter... nal Application No
PCT/EP 00/08895

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
DE 19841337	C	23-09-1999	EP 0962524	A	08-12-1999
			JP 11346764	A	21-12-1999
			DE 19827957	A	09-12-1999
			EP 0960933	A	01-12-1999
			JP 11346794	A	21-12-1999
DE 19827957	A	09-12-1999	DE 19841337	C	23-09-1999
			EP 0962524	A	08-12-1999
			EP 0960933	A	01-12-1999
			JP 11346764	A	21-12-1999
			JP 11346794	A	21-12-1999
DE 19712309	A	20-05-1998	WO 9822819	A	28-05-1998
			EP 0938674	A	01-09-1999
US 5262128	A	16-11-1993	AU 6640190	A	16-05-1991
			EP 0497885	A	12-08-1992
			WO 9105519	A	02-05-1991
WO 9705922	A	20-02-1997	DE 19529371	A	13-02-1997
			EP 0844896	A	03-06-1998
			JP 11511248	T	28-09-1999
			US 6032062	A	29-02-2000
EP 0627621	A	07-12-1994	JP 2641400	B	13-08-1997
			JP 7218510	A	18-08-1995
			US 5489515	A	06-02-1996
			DE 59306529	D	26-06-1997
EP 0689051	A	27-12-1995	CN 1131744	A	25-09-1996
			JP 8062209	A	08-03-1996
			KR 150390	B	01-10-1998
			US 5563067	A	08-10-1996
DE 19646505	A	14-05-1998	DE 59702254	D	28-09-2000
			WO 9820974	A	22-05-1998
			EP 0938383	A	01-09-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

Figure 5
Caption reads: PRIOR ART

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter ...nal Application No
PCT/EP 00/08895

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 19841337 C	23-09-1999	EP 0962524 A	08-12-1999
		JP 11346764 A	21-12-1999
		DE 19827957 A	09-12-1999
		EP 0960933 A	01-12-1999
		JP 11346794 A	21-12-1999
DE 19827957 A	09-12-1999	DE 19841337 C	23-09-1999
		EP 0962524 A	08-12-1999
		EP 0960933 A	01-12-1999
		JP 11346764 A	21-12-1999
		JP 11346794 A	21-12-1999
DE 19712309 A	20-05-1998	WO 9822819 A	28-05-1998
		EP 0938674 A	01-09-1999
US 5262128 A	16-11-1993	AU 6640190 A	16-05-1991
		EP 0497885 A	12-08-1992
		WO 9105519 A	02-05-1991
WO 9705922 A	20-02-1997	DE 19529371 A	13-02-1997
		EP 0844896 A	03-06-1998
		JP 11511248 T	28-09-1999
		US 6032062 A	29-02-2000
EP 0627621 A	07-12-1994	JP 2641400 B	13-08-1997
		JP 7218510 A	18-08-1995
		US 5489515 A	06-02-1996
		DE 59306529 D	26-06-1997
EP 0689051 A	27-12-1995	CN 1131744 A	25-09-1996
		JP 8062209 A	08-03-1996
		KR 150390 B	01-10-1998
		US 5563067 A	08-10-1996
DE 19646505 A	14-05-1998	DE 59702254 D	28-09-2000
		WO 9820974 A	22-05-1998
		EP 0938383 A	01-09-1999

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/EP 00/08895

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 7 G01N33/487 G01N27/403

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 7 G01N

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)
EPO-Internal, WPI Data, PAJ, INSPEC

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	DE 198 41 337 C (MICRONAS INTERMETALL GMBH) 23. September 1999 (1999-09-23)	9-12
Y	Spalte 2, Zeile 20 - Zeile 48 Spalte 3, Zeile 8 - Zeile 12 Spalte 3, Zeile 27 - Zeile 44 Spalte 4, Zeile 27 - Zeile 47 Spalte 5, Zeile 35 - Zeile 41 Spalte 6, Zeile 33 - Zeile 41 Spalte 7, Zeile 32 - Zeile 51 Spalte 8, Zeile 36 - Zeile 65 Spalte 11, Zeile 4 - Zeile 23 Spalte 12, Zeile 28 - Zeile 56 ---	1-8,15, 17,18, 20,21, 23,27

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[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

[X] Siehe Anhang Patentfamilie

* Besondere Kategorien von angegebenen Veröffentlichungen :
"A" Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist
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"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
21. November 2000	12/12/2000

Name und Postanschrift der Internationalen Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Stussi, E

3

Formblatt PCT/ISA/210 (Blatt 2) (Juli 1992)

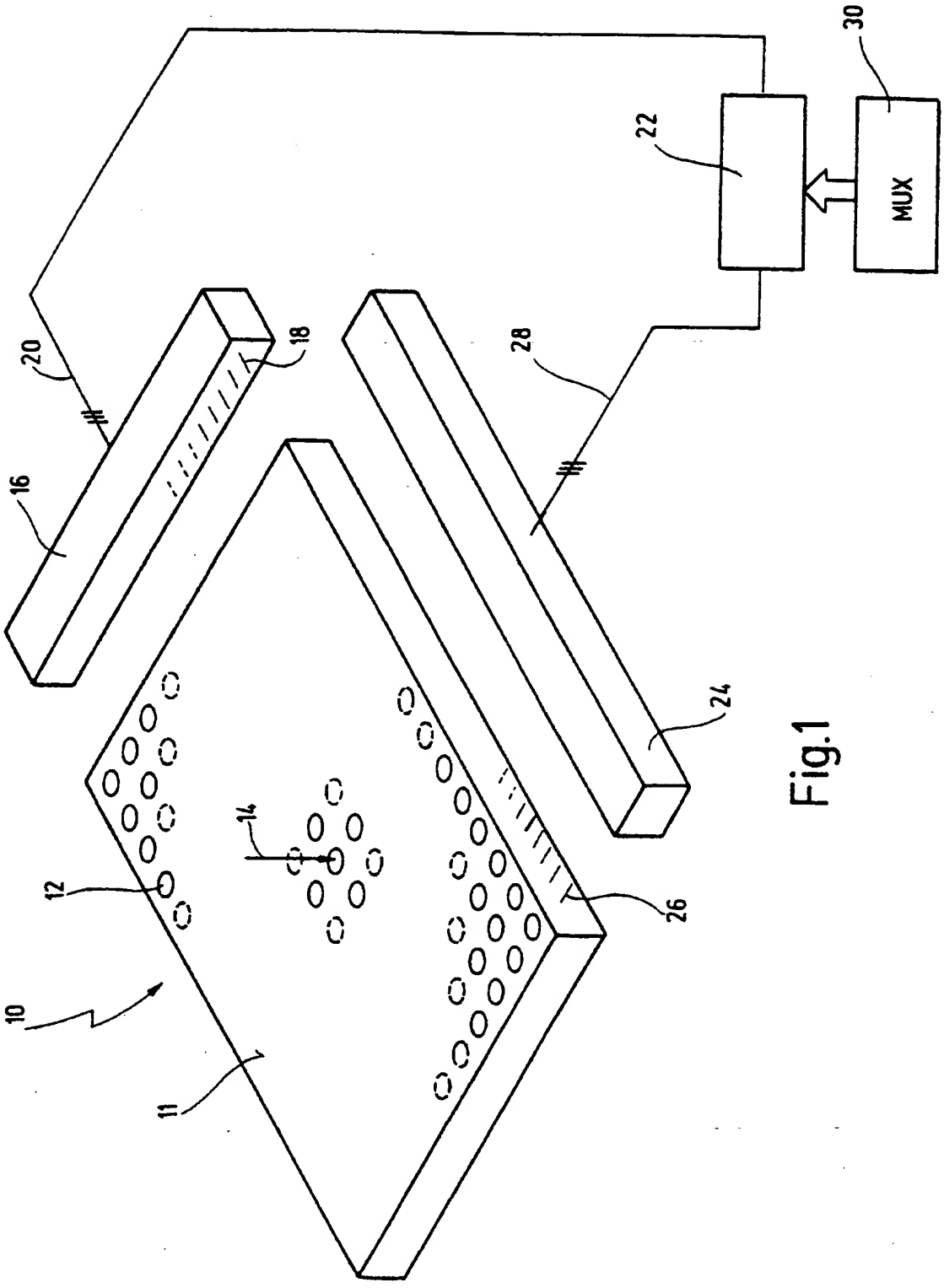

Fig.1

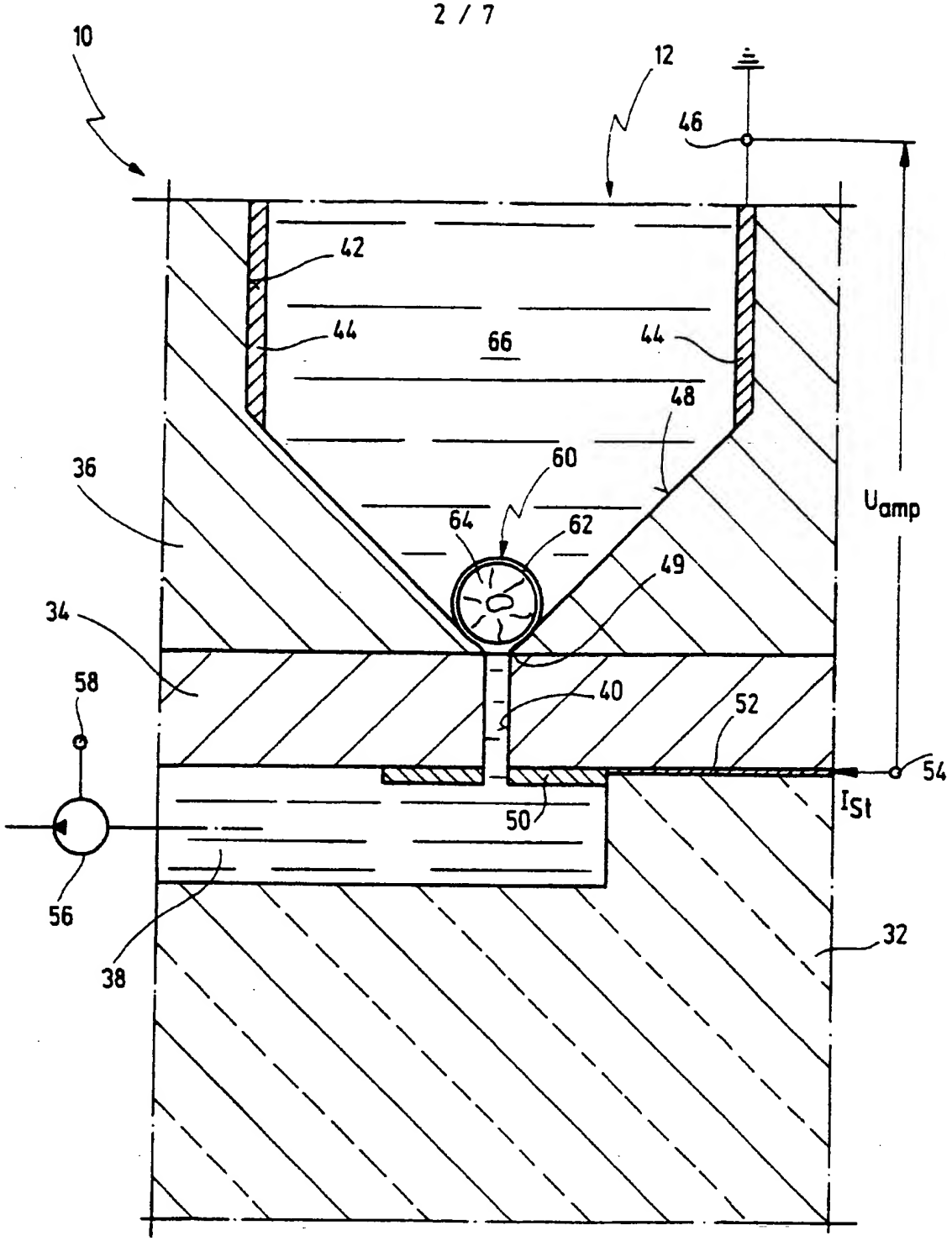
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Fig.2

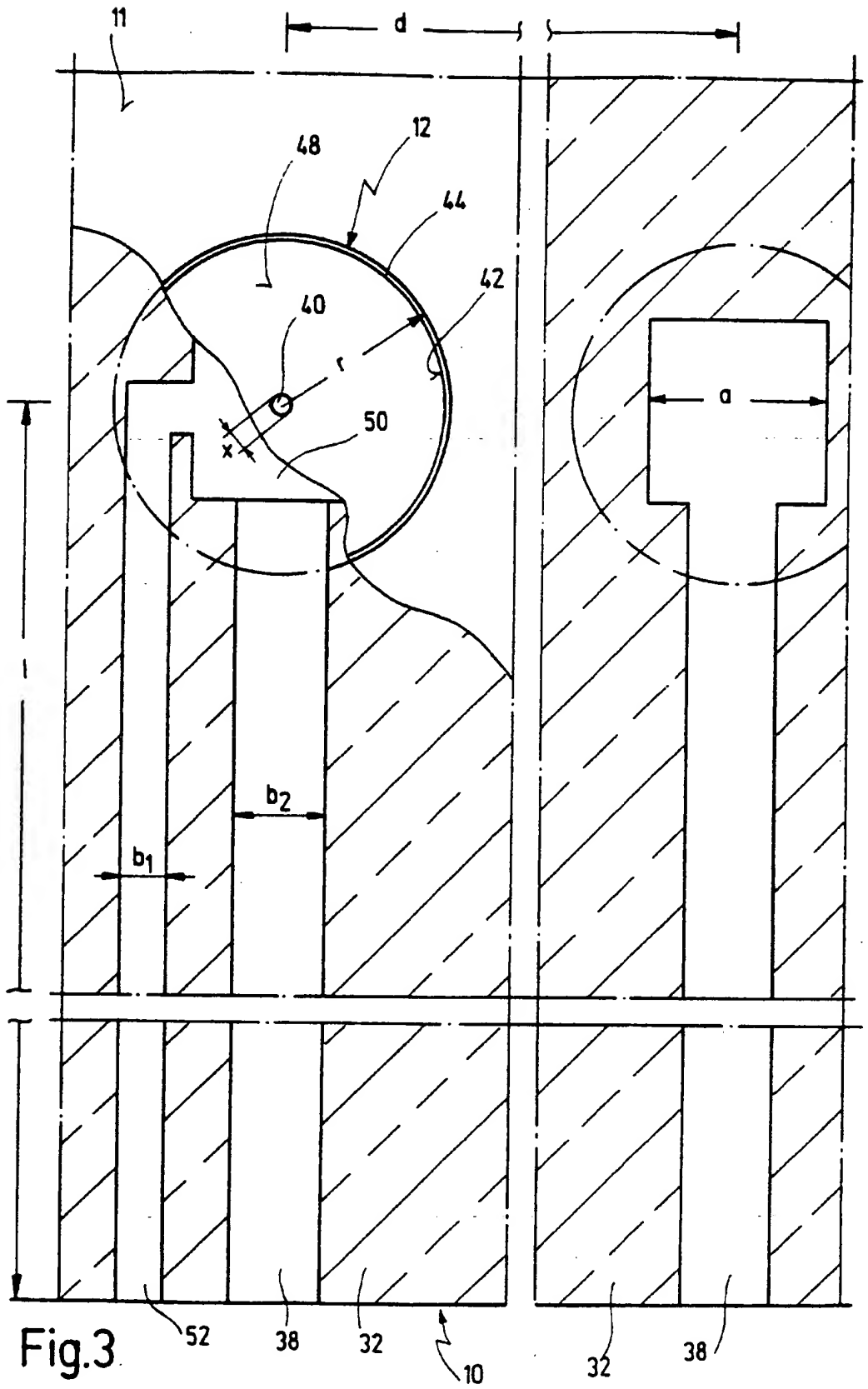


Fig.3

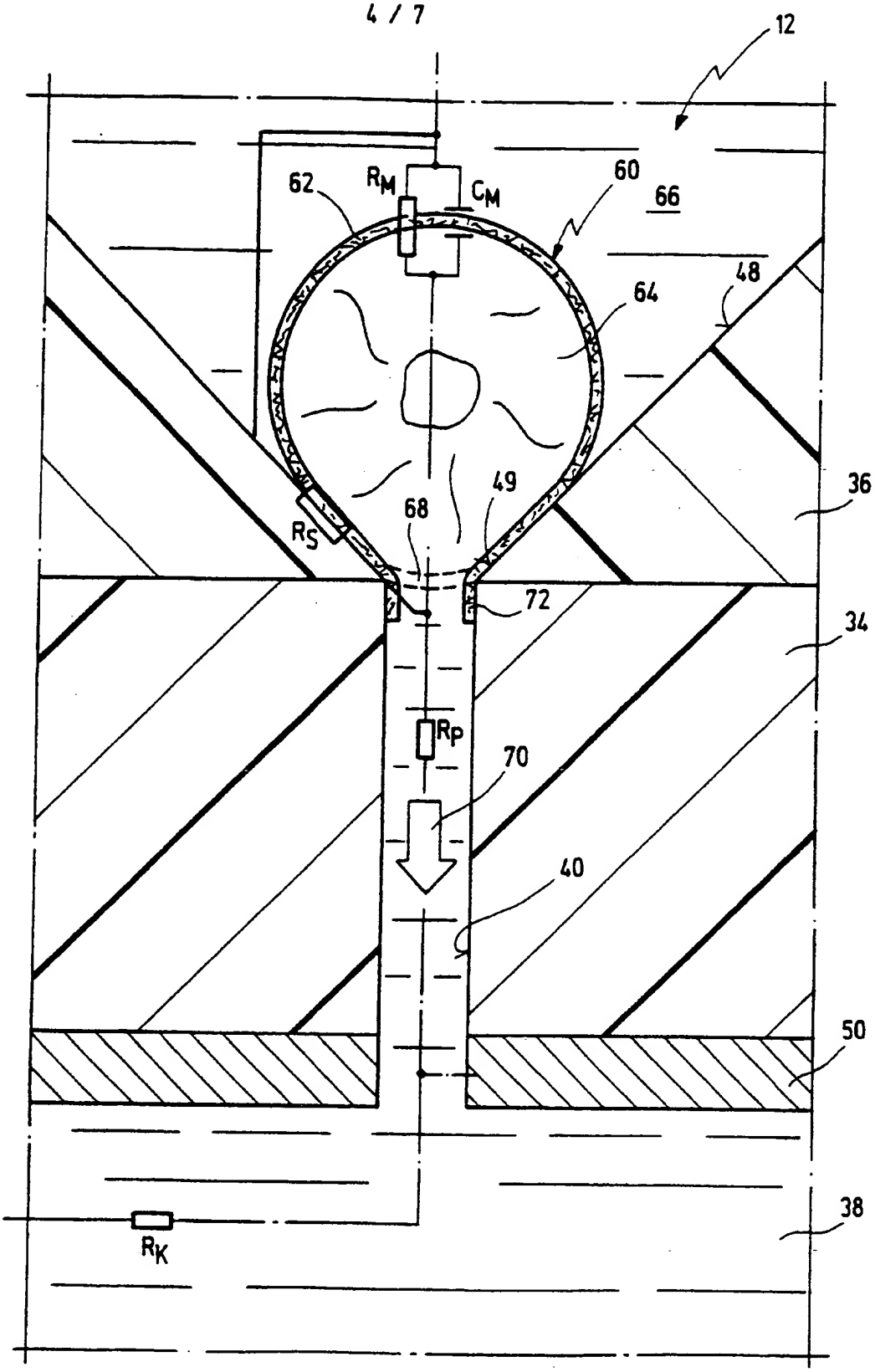
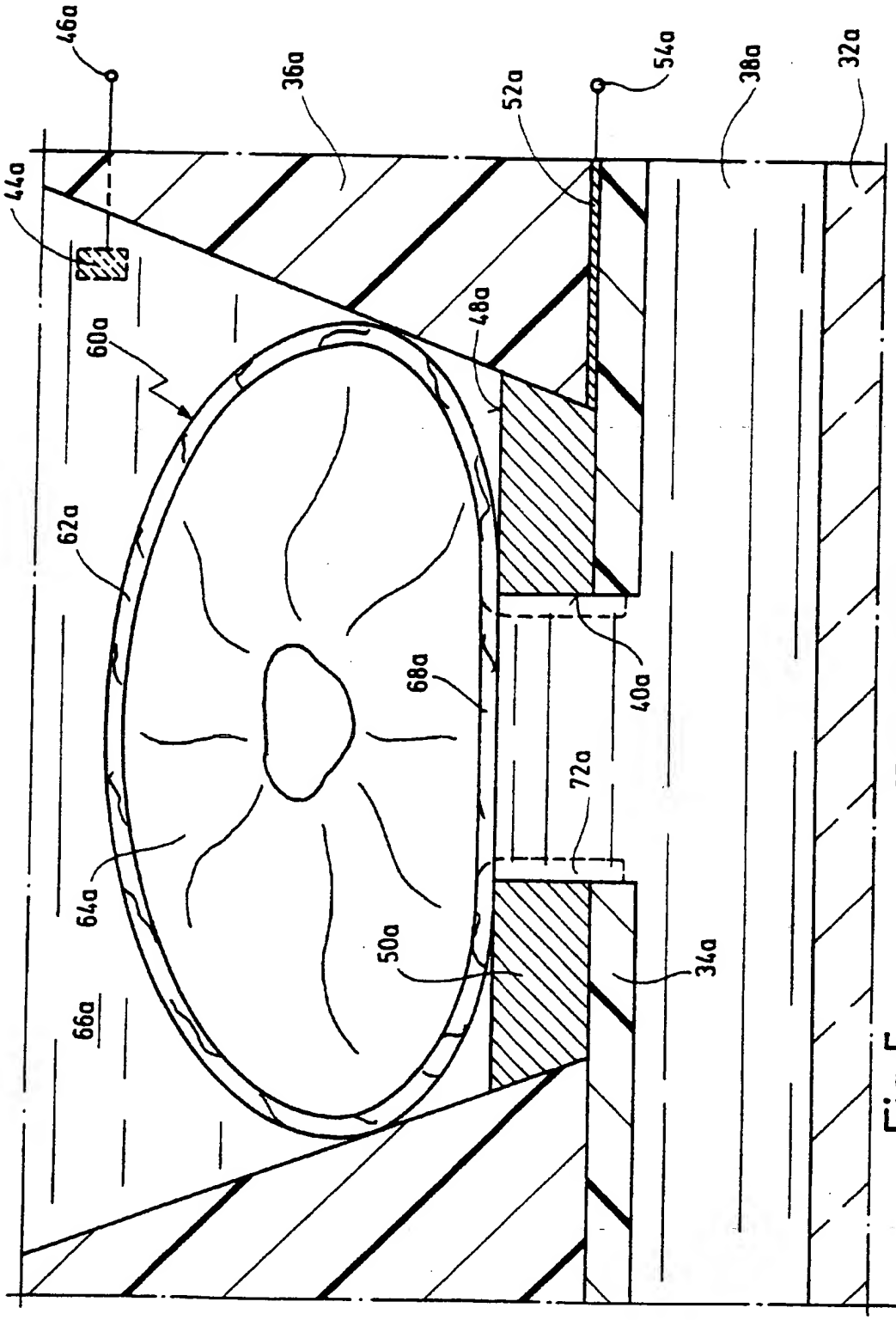


Fig.4



STAND DER TECHNIK

Fig. 5

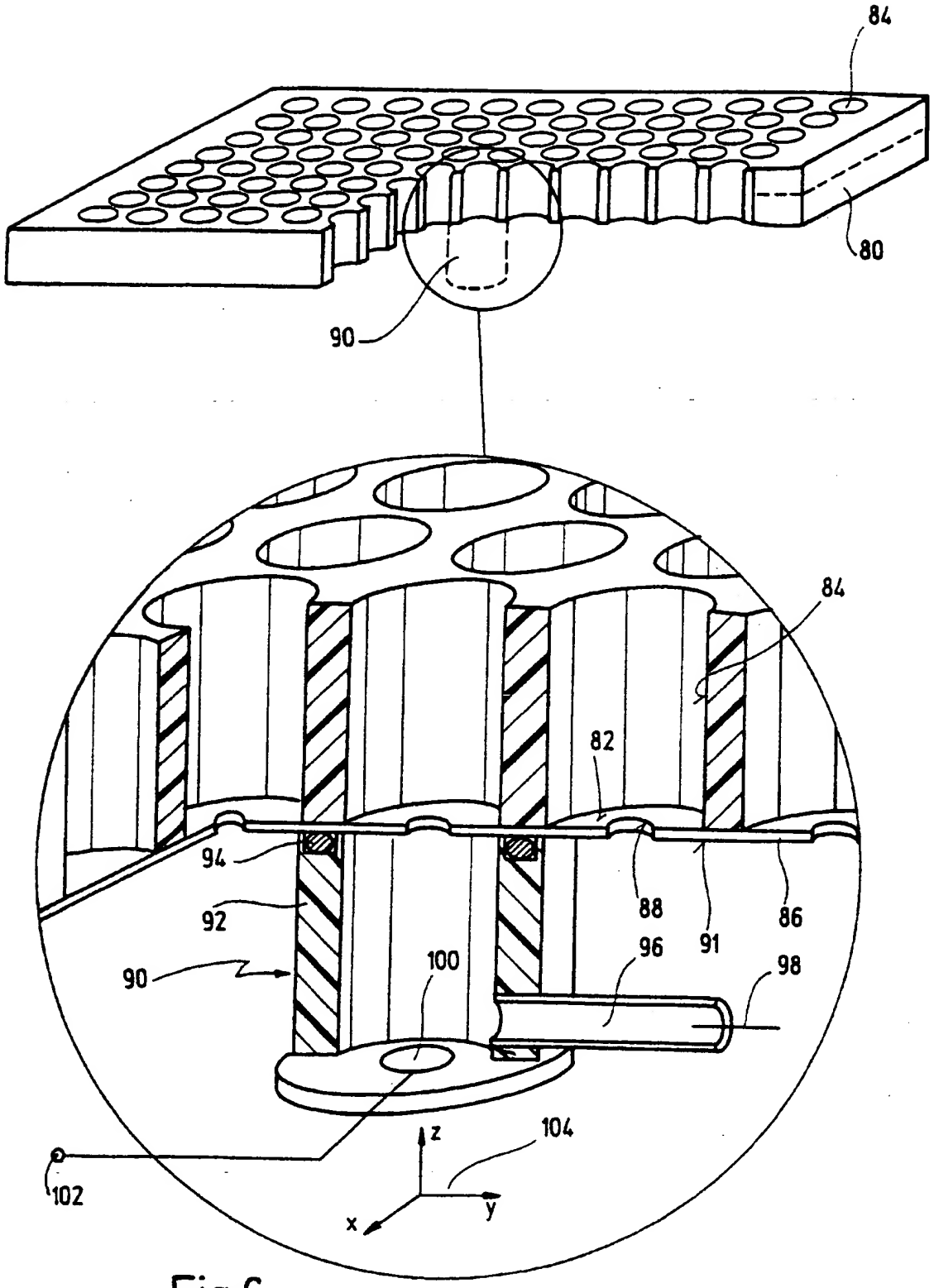
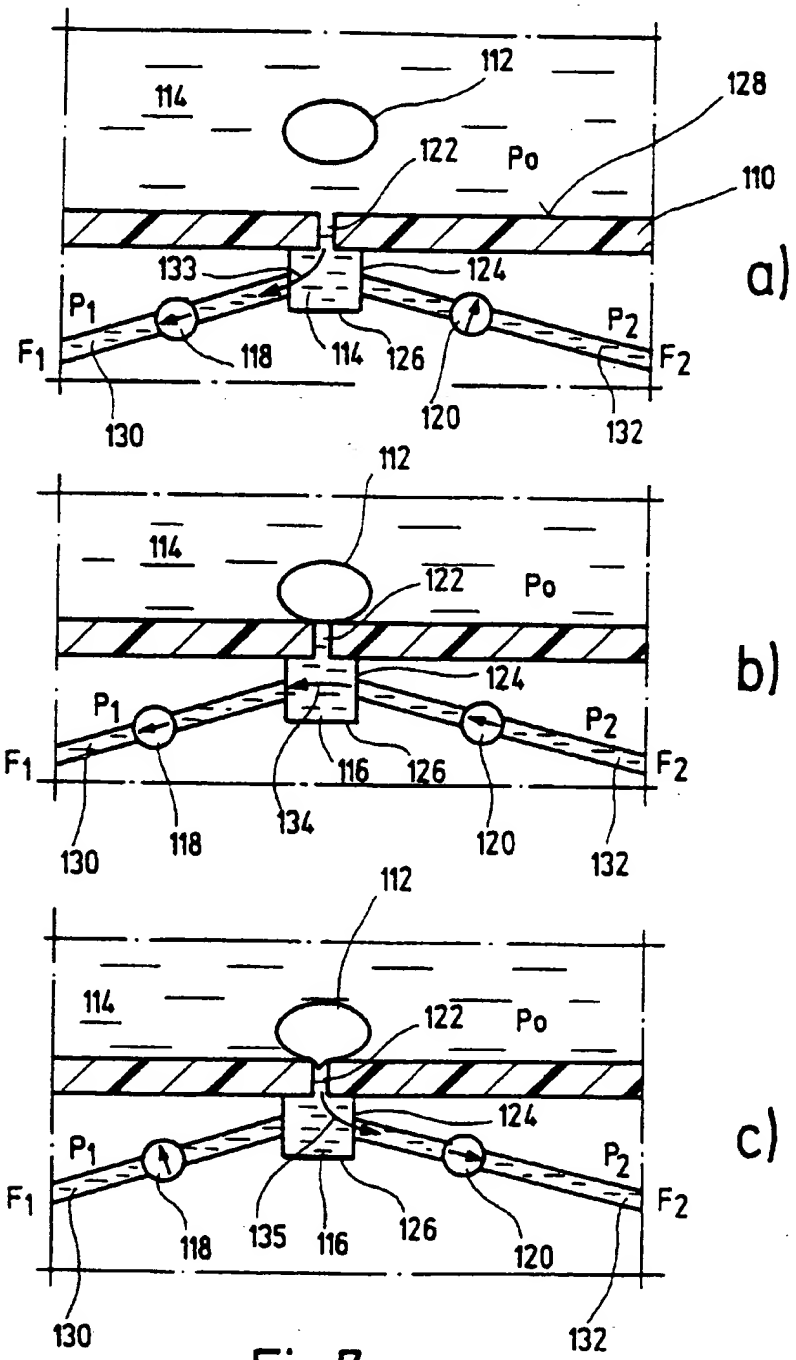


Fig.6


112
114
128
122
Po
110
133
124
a)
P1
P2
F1
F2
118
114
126
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112
114
122
Po
124
b)
P1
P2
F1
F2
116
126
134
130
118
120
132
112
114
122
Po
124
c)
P1
P2
F1
F2
118
135
116
126
120
130
132

Fig.7

INTERNATIONALER RECHERCHENBERICHT

Inter nales Aktenzeichen
PCT/EP 00/08895

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
Y	E. NEHER AND B. SAKMANN: "Single channel currents recorded from membrane of denervated frog muscle fibers" NATURE, Bd. 260, 1976, Seiten 799-802, XP000943702 London Spalte 1 -Spalte 2; Abbildung 1 ---	1,2,4-8
X,P	DE 198 27 957 A (MICRONAS INTERMETALL GMBH) 9. Dezember 1999 (1999-12-09) Spalte 1 -Spalte 4 Spalte 6, Zeile 39 - Zeile 50 Spalte 15, Zeile 68 -Spalte 16, Zeile 42; Abbildung 22 ---	1,2,4,5, 8-12
Y	DE 197 12 309 A (NMI NATURWISSENSCHAFTLICHES UN) 20. Mai 1998 (1998-05-20) in der Anmeldung erwähnt Spalte 4, Zeile 1 - Zeile 10 Spalte 5, Zeile 48 - Zeile 55 Abbildungen 1-3 ---	3,15,17, 18,20, 21,23,27
A	US 5 262 128 A (LEIGHTON STEPHEN B ET AL) 16. November 1993 (1993-11-16) Spalte 2, Zeile 5 - Zeile 41 Spalte 3, Zeile 54 - Zeile 65 Spalte 4, Zeile 25 - Zeile 34 Spalte 4, Zeile 56 -Spalte 5, Zeile 59 ---	13-34
A	WO 97 05922 A (NISCH WILFRIED ;NMI NATURWISSENSCHAFTLICHES UN (DE)) 20. Februar 1997 (1997-02-20) in der Anmeldung erwähnt das ganze Dokument ---	1-34
A	EP 0 627 621 A (AVL MEDICAL INSTR AG) 7. Dezember 1994 (1994-12-07) das ganze Dokument ---	1-34
A	EP 0 689 051 A (MATSUSHITA ELECTRIC IND CO LTD) 27. Dezember 1995 (1995-12-27) das ganze Dokument ---	1-34
A	DE 196 46 505 A (ITT IND GMBH DEUTSCHE) 14. Mai 1998 (1998-05-14) das ganze Dokument -----	13-34

3

INTERNATIONALER RECHERCHENBERICHT

Angaben zu Veröffentlichungen, die zur selben Patentfamilie gehören

Internales Aktenzeichen
PCT/EP 00/08895

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie	Datum der Veröffentlichung
DE 19841337 C	23-09-1999	EP 0962524 A	08-12-1999
		JP 11346764 A	21-12-1999
		DE 19827957 A	09-12-1999
		EP 0960933 A	01-12-1999
		JP 11346794 A	21-12-1999
DE 19827957 A	09-12-1999	DE 19841337 C	23-09-1999
		EP 0962524 A	08-12-1999
		EP 0960933 A	01-12-1999
		JP 11346764 A	21-12-1999
		JP 11346794 A	21-12-1999
DE 19712309 A	20-05-1998	WO 9822819 A	28-05-1998
		EP 0938674 A	01-09-1999
US 5262128 A	16-11-1993	AU 6640190 A	16-05-1991
		EP 0497885 A	12-08-1992
		WO 9105519 A	02-05-1991
WO 9705922 A	20-02-1997	DE 19529371 A	13-02-1997
		EP 0844896 A	03-06-1998
		JP 11511248 T	28-09-1999
		US 6032062 A	29-02-2000
EP 0627621 A	07-12-1994	JP 2641400 B	13-08-1997
		JP 7218510 A	18-08-1995
		US 5489515 A	06-02-1996
		DE 59306529 D	26-06-1997
EP 0689051 A	27-12-1995	CN 1131744 A	25-09-1996
		JP 8062209 A	08-03-1996
		KR 150390 B	01-10-1998
		US 5563067 A	08-10-1996
DE 19646505 A	14-05-1998	DE 59702254 D	28-09-2000
		WO 9820974 A	22-05-1998
		EP 0938383 A	01-09-1999